Protein Tagging Protocol of *Desulfovibrio vulgaris* Genes

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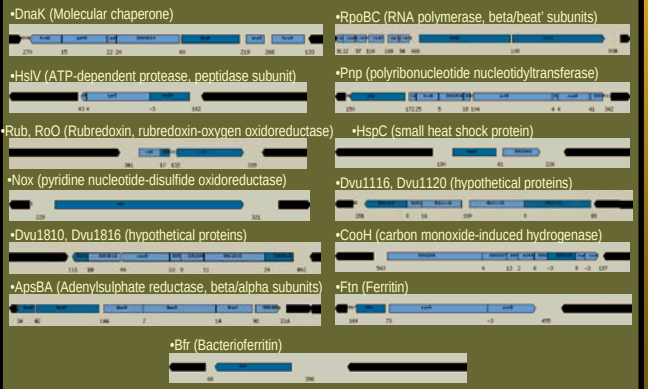
Introduction

The recent development of molecular techniques in *D. vulgaris* is allowing further proteomic studies in this sulfate-reducing bacterium. Included in these techniques is the ability to attach an octapeptide tag for studying protein complexes. The tagged gene is expressed under the wild-type promoter and permits study of protein-complexes without over-expressing one member of the complex more than another. Also, a few variations on the original protocol allow for the tagging of most genes while retaining wild-type expression of the genes involved.

Protocol

- Choose a gene to tag
- Design/order primers (with appropriate restriction sites)
- PCR with Pfu DNA polymerase(Stratagene)
- Capture in TOPO vector (Invitrogen)
- Digest out, ligate into pKASK (modified pASK vector, IBA)
- Verify sequence
- Electroporate into *D. vulgaris*
- Screen for integration by Southern analysis.

Operon Structure of Target Genes

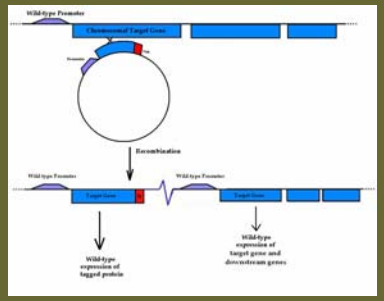


Variations in Technique

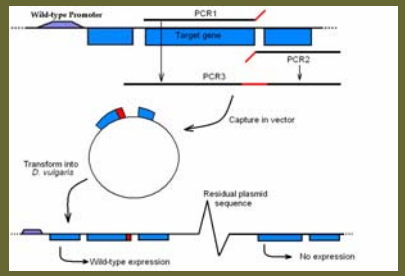
The original protocol requested a *Bsa*I digested PCR product of a gene to be ligated into pKASK that was similarly digested. However, this limits the potential genes for tagging to those lacking internal *Bsa*I sites. In order to create single-copy tagged genes under wild-type promoter control, only promoter-distal or monocistronic genes could be tagged. As a means to circumvent these difficulties, the promoter region could also be duplicated (2) or the remaining portion of the operon could be included on the plasmid following the tagged gene (3). Another option for adding the tag is to PCR the tag onto the gene (1), thereby eliminating the necessity of ligation into pKASK.

1) Place tag on primer. The PCR product will contain the gene with the tag, permitting tagging without reliance on *Bsa*I restriction site.

2) Capture upstream/promoter region of genes at 5' end of operon. Following integration, the entire operon can be expressed under the wild-type promoter, while the tagged version of the protein is also under the wild-type promoter. If the first gene is short enough, the genes immediately down-stream can also be obtained through the same technique.



3) Double PCR for tagging genes near the end of an operon. The tag is built into the primer. First product as #1 above; the second product contains downstream genes to the end of the operon. Second-step of PCR combines first two products and makes a tagged gene in the middle of an operon. Integration of this construct, permits wild-type expression of the tagged gene and all down-stream genes without expression of second copy of gene.



Current Progress

DVU number	Description	PCR	TOPO	pKASK	sequenced	D. vulgaris	Southern
DVU0811	DnaK	x	x	x	x	x	x
DVU2928	RpoB	x	x	x	x	x	x
DVU2929	RpoC	x	x	x	x	x	x
DVU1577	HslV	x	x	x	x	x	x
DVU0503	Pnp	x	x	x	x	x	x
Dvu3185	RoO	x	x	x	x	x	IP
Dvu2441	HspC	x	x	x	IP	IP	
Dvu3184	Rub	x	x	-	x	IP	IP
Dvu3212	nox	x	x	x	x	IP	
Dvu1116	hypothetical	x	x	x	x	IP	
Dvu1120	hypothetical	x	x	x	x	x	IP
Dvu1810	hypothetical	x	x	x	x	x	x
Dvu1816	hypothetical	x	x	x	IP	x	IP
Dvu2291	CooH	x	x	-	x	IP	
Dvu0846	ApsB	x	x	-	x	IP	
Dvu0847	ApsA	x	x	-	x	IP	
Dvu1568	Ftn	x	x	x	x	IP	
Dvu1397	Bfr	x	x	x	x	IP	

(X = completed; IP = In Progress; - = not performed)

Future Work

- Tag unique genes of *D. vulgaris* to determine novel protein complexes.
- Tag hypothetical genes to determine if they are translated and what their funciton is.
- To create a system that allows multiple tags on the same gene for higher-purity protein-complex isolation.

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